

## WHAT IS CLAIMED IS:

1. A compound having the formula

A-B-C-X-Y

5 (I)

or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

B is absent or is from 1 to about 197 naturally-occurring amino acid residues

10 corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

15 R<sup>3</sup> is tyrosyl, 3-I-tyrosyl or phenylalanyl;

R<sup>4</sup> is aspartyl; and

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1 and homologues and analogues thereof.

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2. The compound of Claim 1 wherein B is present and A, C, X and Y are as defined therein.

3. The compound of Claim 2 wherein X is present and A, B, C and Y are as defined therein.

4. The compound of Claim 3 wherein A and Y are present and B, C and X are as defined therein.

5. The compound of Claim 3 wherein A and Y are as defined therein and B-C-X is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;

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10 (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;  
(g) the sequence from amino acid positions 449-546 of SEQ ID NO:1;  
(h) the sequence from amino acid positions 454-546 of SEQ ID NO:1;  
(i) the sequence from amino acid positions 525-535 of SEQ ID NO:1;  
(j) the sequence from amino acid positions 529-535 of SEQ ID NO:1; and  
(k) the sequence from amino acid positions 530-535 of SEQ ID NO:1.

6. The compound of Claim 5 wherein A is N-Ac and Y is -NH<sub>2</sub>.

7. The compound of Claim 1 wherein X is absent and A, B, C and Y are as defined therein.

8. The compound of Claim 7 wherein X, A and Y are as defined therein and B-C is the sequence from amino acid positions 529-534 of SEQ ID NO:1.

9. The compound of Claim 8 wherein A is N-Ac and Y is -NH<sub>2</sub>.

10. The compound of Claim 1 wherein B and X are absent and A, C and Y are as defined therein.

11. The compound of Claim 10 wherein C is the sequence from amino acid positions 531-534 of SEQ ID NO:1.

12. The compound of Claim 11 wherein A is N-Ac and Y is -NH<sub>2</sub>.

13. The compound of Claim 1 wherein said compound has a molecular weight of between 0.5 and 25,000 kilodaltons as determined by reducing polyacrylamide gel electrophoresis or mass spectrometry analysis and an amino acid sequence substantially similar to the corresponding amino acid sequence of SEQ ID NO: 1.

14. The compound of Claim 1 having an endothelial cell migration inhibition ED<sub>50</sub> of about 100 to about 500 pM.

15. The compound of Claim 1 having an endothelial cell proliferation inhibition ED<sub>50</sub> of about 100 to about 500 pM.

16. A compound having the formula

A-B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub>-Y

(II)

or a pharmaceutically acceptable salt, ester or prodrug thereof wherein

5           A is absent or a nitrogen protecting group;  
Y is absent or a carboxylic acid protecting group;  
B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues  
corresponding to the sequence from about amino acid position 334 to amino acid position  
513 of SEQ ID NO:1;

10           C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ  
ID NO:1; and  
X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues  
corresponding to the sequence from amino acid position 524 to amino acid position 533 of  
SEQ ID NO:1 and homologues and analogues thereof.

15           17. The compound of Claim 13 wherein B<sub>1</sub> and X<sub>1</sub> are absent and A, C<sub>1</sub> and Y are as  
defined therein.

18. The compound of Claim 17 wherein A is N-Ac and Y is -NH<sub>2</sub>.

19. A kringle 5 peptide fragment which has substantial sequence homology to a  
plasminogen fragment selected from human, murine, bovine, Rhesus monkey and porcine  
plasminogen.

20. A kringle 5 peptide fragment or fusion protein wherein the kringle 5 peptide  
fragment or kringle 5 fusion protein has a substantial sequence homology to human  
plasminogen.

21. A method of treating a disease in a patient in need of antiangiogenesis therapy  
comprising administering to a human or animal a therapeutically effective amount of a  
mammalian kringle 5 peptide fragment or kringle 5 fusion protein.

22. The method of Claim 21 wherein said mammalian kringle 5 peptide fragment or  
kringle 5 fusion protein is selected from the group consisting of a human, murine, bovine,  
Rhesus monkey and porcine kringle 5 peptide fragment or fusion protein.

23. The method of Claim 22 wherein said kringle 5 peptide fragment or kringle 5 fusion  
protein is a human kringle 5 peptide fragment or kringle 5 fusion protein.

24. The method of Claim 21 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula

A-B-C-X-Y

(I)

5 or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 10 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl, 3-I-tyrosyl or phenylalanyl;

15 R<sup>4</sup> is aspartyl; and

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1 and homologues or analogues thereof.

25. The method of Claim 24 wherein said mammalian kringle 5 fragment or kringle 5 fusion protein is said compound wherein A and Y are as defined therein and B-C-X is selected from the group consisting of

- 5 (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;
- (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;
- 10 (g) the sequence from amino acid positions 449-546 of SEQ ID NO:1;
- (h) the sequence from amino acid positions 454-546 of SEQ ID NO:1;
- (i) the sequence from amino acid positions 525-535 of SEQ ID NO:1;
- (j) the sequence from amino acid positions 529-535 of SEQ ID NO:1; and
- (k) the sequence from amino acid positions 530-535 of SEQ ID NO:1.

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26. The method of Claim 25 wherein A is N-Ac and Y is -NH<sub>2</sub>.

27. The method of Claim 24 said compound is said mammalian kringle 5 fragment wherein X is absent, A and Y are as defined therein and B-C is the sequence from amino acid positions 529-534 of SEQ ID NO:1.

28. The method of Claim 27 wherein A is N-Ac and Y is -NH<sub>2</sub>.

29. The method of Claim 24 wherein said compound is said mammalian kringle 5 fragment wherein X and B are absent, and A, C and Y are as defined therein.

30. The method of Claim 29 wherein A is N-Ac and Y is -NH<sub>2</sub>.

31. The method of Claim 21 wherein said disease is selected from the group consisting of cancer, arthritis, macular degeneration and diabetic retinopathy.

32. The method of Claim 31 wherein said disease is cancer.

33. The method of Claim 32 wherein said disease is selected from primary and metastatic solid tumors, carcinomas, sarcomas, lymphomas, psoriasis and hemangiomas.

34. The method of Claim 21 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula

A-B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub>-Y  
(II)

5 or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position

10 513 of SEQ ID NO:1;

C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of

15 SEQ ID NO:1 and homologues or analogues thereof.

35. The method of Claim 34 wherein said compound is said mammalian kringle 5 peptide fragment wherein B<sub>1</sub> and X<sub>1</sub> are absent, A, C<sub>1</sub> and Y are as defined therein.

36. A composition comprising an isolated single- or double-stranded polynucleotide sequence that encodes a kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

37. The composition of Claim 36 wherein said polynucleotide sequence is a DNA sequence.

38. The composition of Claim 37 wherein said DNA sequence encodes an amino acid sequence selected from the group consisting of

- (a) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 454-543 of SEQ ID NO:1; and
- (d) the sequence from amino acid positions 355-543 of SEQ ID NO:1.

5 39. The composition of Claim 37 wherein said polynucleotide sequence encodes an amino acid sequence selected from the group consisting of SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, and SEQ ID NO:37.

40. A composition comprising a kringle 5 peptide fragment or kringle 5 fusion protein and a pharmaceutically acceptable excipient.

41. A method comprising implanting into a human or non-human animal a cell containing a vector, wherein said vector contains a DNA sequence encoding a kringle 5 peptide fragment or kringle 5 fusion protein and wherein said vector is capable of expressing said kringle 5 peptide fragment or kringle 5 fusion protein when present in said cell.

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42. A method of making a kringle 5 peptide fragment comprising the steps of:

- (a) exposing a mammalian plasminogen to elastase at a ratio of about 1:100 to about 1:300 to form a mixture of said plasminogen and said elastase;
- (b) incubating said mixture; and
- (c) isolating said kringle 5 from said mixture.

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43. An isolated single- or double-stranded polynucleotide which encodes a mammalian kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

44. The polynucleotide of Claim 43 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein encoded by said polynucleotide is selected from the group consisting of human, Rhesus monkey, bovine, murine, and porcine kringle 5 peptide fragment or fusion protein.

45. The polynucleotide of Claim 44 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a human kringle 5 peptide fragment or kringle 5 fusion protein.

46. The polynucleotide of Claim 43 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula B-C-X or B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub> wherein B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

5 C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl or phenylalanyl; and

R<sup>4</sup> is aspartyl;

10 X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1;

B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

15 C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

20 X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID NO:1 and complements thereof.

47. The polynucleotide of Claim 46 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein B is present and C and X are as defined therein.

48. The polynucleotide of Claim 46 wherein said a mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein X is present and B and C are as defined therein.

49. The polynucleotide of Claim 46 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a fragment wherein B and X are present and C is as defined therein.

50. The polynucleotide of Claim 43 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;
- (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;
- (g) the sequence from amino acid positions 449-546 of SEQ ID NO:1; and
- (h) the sequence from amino acid positions 454-546 of SEQ ID NO:1.

5 10 51. The polynucleotide of Claim 46 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a fragment wherein X is absent and B and C are as defined therein.

52. The polynucleotide of Claim 43 which is a DNA molecule.

53. The polynucleotide of Claim 43 which is an RNA molecule.

54. A vector comprising a polynucleotide which encodes a mammalian kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

55. The vector of Claim 57 which is an expression vector.

56. The vector of Claim 57 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein encoded by said polynucleotide is a compound having the formula B-C-X or B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub> wherein

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

10                   R<sup>2</sup> is leucyl or arginyl;  
                  R<sup>3</sup> is tyrosyl or phenylalanyl; and  
                  R<sup>4</sup> is aspartyl;  
                  X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1;

15                   B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amion acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

                  C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

20                   X<sub>1</sub> is absent or is from 1 to about 10 natuually-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID nO:1 and complements thereof.  
                  and complements thereof.

57.           The vector of Claim 56 which is an expression vector.

58.           The vector of Claim 57 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein B and X are present and C is as defined therein.

59.           The vector of Claim 57 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein X is absent and B and C are as defined therein.

60.           The vector of Claim 57 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is selected from the group consisting of

5                   (a)   the sequence from amino acid positions 355-543 of SEQ ID NO:1;  
                  (b)   the sequence from amino acid positions 355-546 of SEQ ID NO:1;  
                  (c)   the sequence from amino acid positions 443-543 of SEQ ID NO:1;  
                  (d)   the sequence from amino acid positions 449-543 of SEQ ID NO:1;  
                  (e)   the sequence from amino acid positions 454-543 of SEQ ID NO:1;  
                  (f)   the sequence from amino acid positions 443-546 of SEQ ID NO:1;  
                  (g)   the sequence from amino acid positions 449-546 of SEQ ID NO:1; and  
10                   (h)   the sequence from amino acid positions 454-546 of SEQ ID NO:1.

61. The vector of Claim 57 selected from the group consisting of pHil-D8, pET32a, pGEX-4T-2, Up-ET, UpET-Ubi, and pCYB3.
62. The vector of Claim 57 further comprising a host cell transformed with said vector.
63. The vector of Claim 62 wherein said host cell is a eukaryotic cell.
64. The vector of Claim 63 wherein said eukaryotic cell is *Pichia pastoris*.
65. The vector of Claim 57 wherein said host cell is a prokaryotic cell which is *E. coli*.
66. A method for making a soluble kringle 5 peptide fragment or kringle 5 fusion protein comprising the steps of:
  - (a) isolating a polynucleotide which encodes said kringle 5 peptide fragment;
  - (b) cloning said polynucleotide into an expression vector;
  - (c) transforming said vector into a suitable host cell; and
  - (d) growing said host cell under conditions suitable for the expression of said soluble kringle 5 peptide fragment or kringle 5 fusion protein.
67. A compound selected from the group consisting of
  - (a) A-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-Y;
  - (b) A-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-Y;
  - (c) A-Pro-Glu-Lys-Arg-Tyr-Asp-Tyr-Y; and
  - (d) A-Gln-Asp-Trp-Ala-Ala-Gln-Glu-Pro-His-Arg-His-Ser-Ile-Phe-Thr-Pro-Glu-Thr-Pro-Glu-Thr-Asn-Pro-Arg-Ala-Gly-Leu-Glu-Lys-Asn-Tyr-Y

68. A compound of Claim 68 wherein A is N-Ac and Y is -NH<sub>2</sub>.